It is not believed that this response occasion's any fee, but should there be any fee, please charge (or credit) the same to Deposit Account No. 02-4467. A duplicate copy of this paper is enclosed.

AMENDMENT

Please amend the application as follows:

In The Specification

Please amend the specification as follows:

Page 1, line 3 (i.e., the line between the title and the BACKGROUND OF THE INVENTION heading) insert the following new heading and paragraph:

--CROSS REFERENCE TO RELATED APPLICATION

This application is a continuation-in-part of United States Serial Number 09/019,932 which is copending. --

In The Claims

Please amend the claims as follows:

1. (Amended) A method for discrimination and counting erythroblasts comprising the steps of:

(i) staining leukocytes in a hematologic sample by adding a fluorescent labeled leukocyte binding antibody [capable of binding specifically with leukocytes] to the hematologic sample;

(ii) raising the permeability only of cell membranes of erythroblasts in the hematologic sample to a nucleotide fluorescent dye which does not permeate a

cell membrane when the permeability is not raised [usually], the nucleotide fluorescent dye having a fluorescent spectrum that is distinguishable [capable of being distinguished] from that of a fluorescent labeling compound of the fluorescent labeled antibody in step (i);

(iii) staining nuclei of the erythroblasts in the hematologic sample with the nucleotide fluorescent dye;

(iv) analyzing [subjecting] the hematologic sample using flow cytometry [flowcytometry] to detect the nucleotide fluorescent signal of the erythroblasts and the fluorescent labeled antibody signal of the leukocytes [at least two fluorescent signals from each cell]; and

(v) discriminating between erythroblasts and leukocytes in the hematologic sample and counting the erythroblasts from a difference in nucleotide fluorescent signal of the erythroblast and the fluorescent labeled antibody signal of the leukocytes [intensity between the two fluorescent signals].

2. (Amended) The [A] method according to claim 1, wherein the fluorescent labeled leukocyte binding antibody [capable of binding specifically with leukocytes] in the step (i) recognizes an antigen present on the leukocytes surface and binds with the antigen.

3. (Twice Amended) The [A] method according to claim 1 wherein labeled leukocyte binding the fluorescent [labeling compound] of the fluorescent labeled antibody in the step (i) comprises at least one compound selected from the group consisting of phycoerythrin, fluorescein isothiocyanate, allophycocyanin, Texas Red, [CY5 stands for a arysulfonate cyanine fluorescent dye, a peridinin chlorophyll

complex, and a combination thereof.

4. (Amended) The [A] method according to claim 1, wherein the raising of the permeability [only] of the cell membranes of erythroblasts in the hematologic sample to the nucleotide fluorescent dye in step (ii) comprises the steps of:

(i) [(1)] admixing a first reagent fluid of hypotonic osmolarity containing a buffer for maintaining pH within an acidic range to the hematologic sample after the step (i); and

(ii) [(2)] admixing thereto a second reagent fluid containing a buffer for neutralizing the first reagent fluid containing the hematologic sample and adjusting a mixture of the hematologic sample and the first reagent fluid to a pH wherein the leukocytes are stained [suitable for staining] and an osmolarity compensating agent for adjusting the mixture to an osmolarity suitable for retaining the shape and integrity of the leukocytes.

5. (Twice Amended) The [A] method according to claim 1, wherein the staining of the nuclei of the erythroblasts in the step (iii) is carried out by mixing the hematologic sample with the nucleotide fluorescent dye.

6. (Amended) The [A] method of claim 5, wherein the nucleotide fluorescent dye comprises at least one compound selected from the group consisting of propidium iodide, N-methyl-4-(1-pyrene)-vinyl-propidium iodide, ethidium bromide, TOTO-1, TOTO-3, YOYO-1, YOYO-3, BOBO-1, BOBO-3, ethidium homodimer-1, ethidium homodimer-2, POPO-1, POPO-3, BO-PRO-1, YO-PRO-1 and TO-PRO-1.

7. (Amended) The [A] method according to claim 1, wherein the nucleotide fluorescent signal of the erythroblasts and the fluorescent labeled antibody signal of the leukocyte [at least two fluorescent signals detected from each cell includes a fluorescent signal based on the fluorescent labeled antibody capable of binding specifically with leukocytes and a fluorescent signal based on the nucleotide fluorescent dye and the two fluorescent signals] are plotted in two coordinate axes to obtain a two-dimensional distribution chart.

8. (Twice Amended) The [A] method according to claim 1, wherein an area in which the erythroblasts appear is defined on the two dimensional distribution chart and the number of erythroblast cells in the area is connected.

9. (Twice Amended) The [A] method according to claim 1, wherein areas in which the leukocytes and the erythroblasts appear are defined on the two-dimensional distribution chart, the number of cells in each of the areas is countered to obtain a leukocyte count and an erthyroblast count, and the erythroblast count is divided by the leukocyte count, whereby the ratio of erythroblasts to leukocytes is obtained.

10. (Amended) The [A] according to claim 5, wherein the nucleotide fluorescent dye is used at a concentration within the range of 0.003mg/L to 10mg/L to form a [in a] mixture to be analyzed [subjected] using flow cytometry [to flowcytometry] to stain erythroblasts according to degrees of maturity of the erythroblasts, and thereby the erythroblasts are classified into at least two groups according to the degrees of maturity thereof.

11. (Amended) The [A] method according to claim 10, wherein:

(i) [(1)]the nucleotide fluorescent signal of the erythroblasts and the fluorescent labeled antibody signal of the leukocytes [at least two fluorescent signals detected for each cell includes a fluorescent signal based on the fluorescent labeled antibody capable of binding specifically with leukocytes and a fluorescent signal based on the nucleotide fluorescent dye and the two fluorescent signals] are plotted in two coordinate axes to obtain a two-dimensional distribution chart;

(ii) [(2)] areas are set in the two-dimensional distribution chart for classifying erythroblasts into at least two groups from difference in intensity of the fluorescent signals based on the nucleotide fluorescent dye; and

(iii) the number of cells in each of the areas is counted for obtaining counts of erythroblasts at different degrees of maturity.

12. (Amended) The [A] method of according to claim 11, wherein an area of all erythroblasts and areas of at least two groups of erythroblasts at different degrees of maturity are defined in the two-dimensional distribution chart, the number of cells in each of the areas is counted to obtain a [an] total erythroblast count and counts of erythroblasts at the respective degrees of maturity, and the counts of erythroblast at the respective degrees of maturity are divided by the total erythroblast count, whereby the ratios of the erythroblasts at the respective degrees of maturity to all the erythroblasts are obtained.

REMARKS

This is in response to the office action mailed September 28, 1999 and resent March 28, 2000. As requested by the Examiner the specification has been